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Peripheral B-cell maturation: the intersection of selection and homeostasis

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Summary: B cells complete maturation after migrating to the periphery, where they transit several intermediate developmental stages prior to recruitment into the long-lived primary pool. Because B-lineage commitment is not regulated by peripheral pool size and most peripheral B cells are quiescent, the primary factors governing steady-state numbers are the proportion of immature B cells surviving transit through later developmental stages and the longevity of mature B cells themselves. Substantial evidence indicates that the B-cell receptor (BCR) plays an essential role in all these processes, but recent findings suggest a central role for the recently described tumor necrosis factor (TNF) family member, B-lymphocyte stimulator (BLyS). Signaling through one of the BLyS receptors, BLyS receptor 3 (BR3), controls B-cell numbers in two ways: by varying the proportion of cells that complete transitional B-cell development and by serving as the primary determinant of mature B-cell longevity. The recent discovery that BCR signaling is selectively coupled to BR3 expression in a developmentally regulated fashion links BCR- and BLyS-mediated events, suggesting that specificity-based selection and survival may be mechanistically similar processes.

Lymphocyte homeostasis

Lymphocyte homeostasis poses a unique biological problem, because it calls for balancing the need for a highly diverse and protective repertoire with the avoidance of pathogenic autoreactivity. The notion of monospecificity imposed by clonal selection implies that a diverse array of preimmune clonotypes must be maintained to afford the selectivity observed in adaptive immune responses. In this regard, B-lymphocyte homeostasis might be perceived as an uncomplicated steady-state problem, whereby a naïve B-cell pool of adequate size, and, hence receptor diversity, is sustained by a fixed B-cell lifespan superimposed on a constant production rate. This simple view is precluded, however, by the gene rearrangement strategy employed to achieve receptor diversity. Given the probability of generating either ineffectual or self-reactive receptors through this random process, the straightforward regulation of cell numbers via fixed production

rates and uniform cellular lifespan seems prone to failure and incompatible with acquired tolerance. Instead, selective processes based on antigen receptor specificity determine the relative fitness of clonotypes, both from the standpoint of potential untoward autoreactivity as well as likely protective utility.

The integration of these processes, homeostasis on one hand and specificity-based selection on the other, has proven to be a complex and difficult puzzle for several reasons. First, the identity, lineage relationships, anatomic sites, and kinetics of key differentiative intermediates in the B lineage have been appreciated only in the last decade, and as evidenced by the spectrum of views in this volume, many of these remain debated. Second, the principal molecular mediators of B-cell viability and lifespan have only recently been revealed. Accordingly, this article begins with an overview of the identity and dynamics of these major differentiative subsets, as well as the evidence for specificity-based selection within these populations. The remainder focuses on the role of BLyS and its receptors in these processes. Finally, a model that unifies positive selection and competition amongst naïve follicular B cells under a single mechanistic process is proposed.

The identity and dynamics of follicular B-cell progenitors

In normal adults, early B-cell differentiation ensues in the bone marrow, where successive developmental stages have been defined according to surface marker expression and immunoglobulin (Ig) gene rearrangement status (1–8). These stages and processes are reviewed extensively elsewhere (9–11), but as the immediate predecessors of differentiative stages discussed herein, they are very briefly summarized (*Table* 1). In the pro-B stage (1–3), cells rearrange their heavy-chain genes. This stage can be divided into three fractions according to Hardy (12, 13). Fraction (Fr) A contains cells whose Ig heavy- and light-chain genes are still in the germline configuration, whereas fractions B and C contain cells which have already rearranged D–J_H but not V_H–DJ_H. Successful heavy-chain gene rearrangement heralds the pre-B-cell stage, characterized by surface expression of the pre-B-cell receptor (pre-BCR) with the Igα and Igβ transmembrane signaling molecules. Following a proliferative burst (Fr C′), light-chain gene rearrangement ensues (Fr D). Successful light-chain rearrangement and surface expression of a BCR identify the immature marrow B cell or Hardy fraction E. These cells and their counterparts in the periphery are the immediate progenitors of most mature peripheral B cells. Thus, transit from these pools is the *de facto* production rate of mature peripheral B cells. Furthermore, inasmuch as this point is the first where a complete BCR is expressed, this and all subsequent differentiative stages are potentially subject to specificity-based selection. Finally, this is the first stage where B-lymphocyte stimulator (BLyS) binding is evident (see below), marking the first point at which this cytokine can influence homeostatic processes.

Most B cells complete maturation in the periphery

Contrary to previously held models of B-cell development, it is now appreciated that most newly formed B cells complete maturation after migrating to the periphery (14, 15). Subsequent to their initial description based on heat-stable antigen (HSA)/CD24 intensity (14, 15), these peripheral developmental intermediates were dubbed as 'transitional' cells (16) and have been further subdivided based on two partially overlapping strategies (*Table* 2). In the first, Loder *et al.* (17) forwarded the notion that transitional differentiation proceeds in a stepwise fashion, using CD23, CD21, and sIgD expression to define two transitional categories, termed T1 and T2. A more recently reported strategy devised by Allman *et al.* (18, Allman, this volume) resolves three transitional subpopulations, termed T1, T2, and T3, on the basis of AA4.1, CD23, and sIgM expression. Whilst the T1 pools defined by these two phenotyping strategies are largely congruent, a critical disparity exists in the later stages: in the two-stage scheme, the T2 population is cycling; and in the AA4.1-based scheme, none of the subsets are cycling. The relationship of cycling T2 cells defined by Loder *et al.* (17) to the non-dividing AA4.1^+ T2-3 pools defined by Allman *et al.*

***Table* 1. B-cell subsets and dynamics in mouse bone marrow**

Osmond[a]	Hardy[b]	Production rate[a] (cells/d × 10^{-6})	Cycling	Ig gene status[a,b]	Surface phenotype[b]
Early pro	A	2.5	Yes	Germline	$CD19^-B220^{lo}CD43^+HSA^-AA4.1^+$
Intermed pro	B	5.0	No	D–J_H	$CD19^+B220^{lo}CD43^+HSA^+A4.1^+$
Late pro	C	36	No	V_H–DJ_H	$CD19^+B220^{lo}CD43^+HSA^+AA4.1^+$
Large pre	C′	35	Yes	cyt mu, cyt mu	$CD19^+B220^{lo}CD43^-HSA^+AA4.1^-preBcR^{+c}$
Small pre	D	17	No	V_k–J_k	$CD19^+B220^+HSA^+sIgM^-$
Immature	E	16	No	$sIgM^+sIgD^-$	$CD19^+B220^+HSA^+$

[a]After Osmond DG (2).
[b]After Hardy RR, *et al.* (6), and Li Y-S, *et al.* (7).
[c]After Melchers F, *et al.* (122).

Table 2. Peripheral B-cell subsets and dynamics

Status	Subset	Production rate[a] (cells/d × 10^{-6})	Cycling	Surface phenotype
Transitional[b]				
	T1	1.5	No	$IgM^{hi}CD23^{-}B220^{+}AA4.1^{+}$
	T2		No	$IgM^{hi}CD23^{+}B220^{+}A4.1^{+}$
	T3		No	$IgM^{lo}CD23^{+}B220^{+}AA4.1^{+}$
Transitional[c]				
	T1	ND	No	$IgM^{hi}CD23^{-}$ $CD21^{+}B220^{+}IgD^{-}$
	T2	ND	Yes	$IgM^{lo}CD23^{-+}CD21^{hi}B220^{+}IgD^{+}$
Mature				
	FO (B2)	0.5	No	$IgM^{lo}CD23^{+}B220^{hi}AA4.1^{-}$
	MZ	ND	No (?)	$CD9^{+}IgM^{hi}IgD^{lo}CD23^{-}CD21^{+}$
	B1	ND	Yes (?)	$IgM^{hi}CD43^{+}IgD^{lo/-}CD23^{lo/-}$

FO, follicular B cells; MZ, marginal zone B cells; Ig, immunoglobulin; ND, not determined.
[a]From Allman DM, *et al.* (15).
[b]According to Allman DM, *et al.* (18).
[c]According to Loder F, *et al.* (17).

(18) remains unclear. It is possible that each phenotypically defined subset is an intermediate stage in the final maturation of recent marrow émigrés. Alternatively, one or more of these subsets could represent a compartment outside the pathway of follicular B-cell generation from marrow progenitors. We have adopted the phenotyping strategy of Allman *et al.*, based on recent and ongoing studies suggesting that the subsets defined through this approach most accurately represent the differentiative intermediates of recent marrow émigrés.

Production and turnover rates at the marrow–periphery interface suggest cell losses

Production rate and cellular lifespan are the key determinants of pool size at steady state; hence, measurements of these factors are essential to understanding underlying homeostatic processes. These two parameters can be measured by determining the number of cells generated per unit time and the proportion of a pool replaced per unit time. Moreover, once progenitor–successor relationships are established, differences in the production rates (cells generated per unit time) of a progenitor vs. successor pool yield an estimate of differentiative success; disparities between the number of cells entering a progenitor pool versus those entering the successor pool must be attributed to losses incurred either through cell death or through differentiation into an alternative successor subset. Accordingly, considerable effort has been devoted to examining the production and turnover rates of lymphocyte subpopulations, primarily through *in vivo* labeling strategies.

Assessments of lymphocyte population dynamics were first made by Everett *et al.* (19), using *in vivo* labeling with ^{3}H-thymidine followed by *ex vivo* autoradiography and microscopic enumeration. These experiments as well as extensive studies by Osmond and others (20–22) established two principles. First, they showed that some lymphocytes exhibited a high turnover rate, acquiring label within several days, whereas others required protracted ^{3}H-thymidine administration before labeling. Second, they showed that the rapidly labeling cells predominated in bone marrow and thymus, whereas the secondary lymphoid organs contained cells with slow turnover rates. Together, these findings indicated that lymphocytes are continuously produced and that the mature pool is slowly but continually replenished with newly formed cells. More recently, studies combining *in vivo* bromodeoxyuridine (BrdU) labeling with cytofluorimetric enumeration have confirmed the basic observations that newly formed lymphocytes turn over rapidly, whereas mature cells show slower turnover, indicating a lengthy average lifespan (23, 24).

The application of BrdU labeling and cytofluorimetry to measure production and turnover at the marrow–periphery interface revealed substantial disparities in the production rates between transitional cells versus mature follicular B cells (15). These findings, included in *Tables 1 and 2*, have been confirmed and extended by others, using alternative or more recent phenotyping strategies (18, 25). A key feature is that the rate at which newly formed cells enter the mature pool accounts for less than 5% of the immature (Fr E) cells produced in the bone marrow, suggesting significant cell losses within the transitional populations. These losses appear to occur both at the immature marrow stage and during transit of immature stages in the periphery.

Implications of an immature peripheral intermediate with high-attrition rate

The discovery of an intermediate differentiation stage in the periphery, coupled with kinetic studies indicating that

substantial cell losses occur as cells move through this stage to the mature pool, carried several implications. First, the rate of successful transit through these pools would dictate the production rate of mature B cells and, in conjunction with mature B-cell lifespan, govern peripheral pool size. Second, this compartment would likely be the site of specificity-based selection, both negative and positive, inasmuch as it spans antigen receptor acquisition with entrance to the mature follicular pool.

Transitional success determines mature B-cell production rate

The production rate of mature B cells along with the lifespan of mature B cells themselves are the key determinants of steady-state pool size. Thus, the factors that control production rate are key elements of peripheral B-cell homeostasis. Considerable data suggest that neither commitment to the B lineage nor successful passage through early marrow differentiative stages (Frs A–D) is tied to peripheral B-cell numbers. For example, neither the elimination of peripheral cells with anti-Ig treatment nor the unusually low numbers of mature cells found in various mutant mice yield changes in pro-B-cell generation rates (26–30). Accordingly, mature B-cell production rates are primarily a reflection of the degree to which immature marrow cells successfully navigate the transitional pools.

Negative selection occurs during transitional differentiation

The likelihood that cells within the immature peripheral compartments are subject to negative selection was strongly suggested by several aspects of their original description (14, 15). For example, responses to a variety of *in vitro* stimuli were similar to those of other putative 'immature' B-cell sources. They failed to proliferate under either anti-Ig-mediated BCR cross-linking or phorbol myristate acetate (PMA)/calcium ionophore and yet were responsive to lipopolysaccharide (LPS). Furthermore, these pools contained progenitors capable of primary humoral responses, if T-cell help was provided. Together, these and subsequent observations (31) have suggested that as cells move through successive transitional stages, the results of BCR ligation become increasingly characteristic of maturity (32, 33). Furthermore, it has recently been reported that transitional B cells may be less effective in antigen presentation to helper T cells (34).

Contemporaneous with the description of an immature developmental intermediate in the periphery, Carsetti *et al.* (16) designated bone marrow B cells bearing an identical HSA and sIg phenotype 'transitional' cells. They showed that these were targets of negative selection, furthering the chances that negative selection might extend to the phenotypically similar subset in the periphery.

The Basten laboratory (35) was the first to directly demonstrate negative selection amongst newly formed peripheral B cells *in vivo*. These elegant experiments employed the soluble hen egg lysosome (HEL)/anti-HEL transgenics (36), and they showed that while HEL-binding cells entered the periphery, most died rapidly (approximately 3d) and did not complete maturation. Further substantiating these conclusions was their observation that only cells with endogenous Ig gene rearrangements that eliminated significant HEL binding enjoyed maturation and lengthy lifespan.

Repertoire shifts accompany transitional differentiation

A somewhat more controversial conjecture made upon discovery of the immature peripheral compartment was that B cells awaiting BCR-mediated positive selection would reside in this pool (15). One prediction of such specificity-based positive selection is that the repertoire should truncate appreciably in successive developmental stages where such selection occurs.

The notion of a BCR-mediated selection step in B-cell maturation was first forwarded by Gu *et al.* (37), in studies where V_H gene repertoires of immature (marrow Fr E) versus mature (peripheral and bone marrow Fr F) B-cell subsets were compared. Whilst V_H genes utilized by immature cells were distributed stochastically across the J558 family, the recirculating mature B cells appeared to preferentially utilize two related J558 family genes. The first direct interrogation of whether repertoire shifts commensurate with a specificity-mediated selection step occur between the transitional and mature peripheral B-cell pools resulted from collaborative studies in the Janeway and Shlomchick laboratories. In these investigations, Levine *et al.* (38) demonstrated a skewing of light-chain V gene usage between the transitional and mature peripheral compartments of heavy-chain transgenic mice. Taken together, these findings are most consistent with a selection of clonotypes based on BCR specificity that begins at marrow Fr E and continues through transitional differentiation in the periphery.

Transitional success and mature B-cell survival are active processes

Although the notion that negative selection acts on immature B lymphocytes has been relatively well established and accepted, whether final differentiation to the mature B-cell pool is a 'default' result for cells that avoid negative selection

or instead requires further signals has been less clear. The mounting evidence of repertoire shifts suggesting the latter have paralleled additional observations indicating a positive role for BCR signaling during late differentiation. For example, alterations in BCR-signaling pathways or specificity have yielded either altered transitional differentiation (39–41) or an impaired ability to survive in the presence of signaling-sufficient competitors (42–44).

Thus, the notion of 'positive selection' amongst B lymphocytes has gained momentum during the last several years, such that peripheral developmental intermediates at the marrow periphery interface are now viewed as a triage point from which newly formed cells are either sorted into functional subsets or discarded (45–50). Whilst the exact mechanisms remain the focus of intense investigation (this volume), this triage process seems tightly associated with BCR signaling and specificity.

Mature B-cell populations rely on BCR expression and specificity-dependent events for their survival and competitive advantage

Similar to the competitive processes active during transitional differentiation, relative fitness for survival amongst mature naïve B cells is also dictated by BCR specificity. Indeed, the BCR clearly plays a major role in maintaining the viability of most mature B cells. For example, conditional ablation of BCR expression subsequent to the establishment of steady-state B-cell numbers results in rapid death of most peripheral B cells, and faulty BCR signaling prevents normal B-cell development (51, 52). The exact mechanism through which BCR signaling influences B-cell longevity remains to be resolved, although it appears that basal signaling via the BCR is sufficient to afford some level of survival (53). However, analogous to its role in transitional B-cell selection, BCR specificity also appears pivotal in establishing the relative fitness for survival amongst mature peripheral B cells. This role has been amply demonstrated through experiments employing mixed marrow chimeras and parabiotic approaches from the Freitas laboratory (54–57, see also Freitas, this volume). An important aspect of these studies is the idea that mature B cells appear to be competing for lifespan promoting factor(s) and that their relative competitive advantage is dictated by their BCR specificity.

The A/WySnJ mutation defines a gene controlling peripheral B-cell differentiation and survival

Animals with genetic defects yield insights into many normal processes, and the A/WySnJ mouse has proven to be an informative model for peripheral B-cell maturation and homeostasis. Early studies by Miller *et al.* (58, 59) showed that A/WySnJ mice have one-tenth of the normal number of peripheral B cells but normal numbers of T cells. Furthermore, A/WySnJ mice make primary humoral responses to both TI and TD antigens but poor secondary IgG responses. A single, autosomal co-dominant locus termed 'B-cell maturation defect' (Bcmd), which was eventually mapped to murine chromosome 15, controls this phenotype (30, 59). Two findings have proven key to understanding the biological nature of this lesion. First, the splenic B-cell pool in A/WySnJ expands normally until approximately 3 weeks of age, when it plateaus at an abnormally low level, suggesting failed late differentiation and an inordinately high cell-loss rate in the periphery. Second, bone marrow B-cell numbers and turnover rates are normal in the A/WySnJ, whereas amongst all peripheral differentiation subsets except B1, turnover rates are higher and production rates are lower (30, 60, 61). Key features of this phenotype are summarized in *Table* 3.

***Table* 3. B-cell production and turnover in A/J and A/WySnJ mice[a]**

			Spleen[b]		
	Strain	Bone marrow (Fr E)	T	FO	B1
Total B cells ($\times 10^{-6}$)					
	A/J	32	7.0	48	0.90
	A/WySnJ	32	1.5	3.7	0.99
Production rate (cells/day $\times 10^{-6}$)					
	A/J	9.3	0.7	0.3	0.04
	A/WySnJ	9.3	0.3	0.05	0.04
Turnover rate (%/day)					
	A/J	35	20	0.6	4.1
	A/WySnJ	35	33	2.0	3.6
Average half-life (days)					
	A/J	2.4	7	115	16.7
	A/WySnJ	2.4	5	51	17.6

[a]Data shown are summarized from Lentz *et al.* (30, 60, 61).
[b]Splenic subsets examined include follicular (FO), transitional (T), and B1.

Taken together, these findings showed that the mutated locus in A/WySnJ controls a B-lineage-specific pathway critical for both the recruitment of newly formed B cells into the long-lived peripheral pool as well as for naïve B-cell survival *per se*. Furthermore, these findings were consistent with the idea that both peripheral maturation and longevity rely on discrete ongoing events, rather than simply the avoidance of negative selection. Finally, the observation that a single mutation influenced both transitional maturation and mature cell lifespan suggested that these two parameters might be mechanistically related. The gene product of Bcmd has recently been revealed to be a critical member of the BLyS receptor family (62, 63), identifying BLyS signaling through this receptor as a central regulator of peripheral B-cell homeostasis.

BLyS and its receptors govern peripheral B-cell homeostasis

BLyS, its receptors, and its related cytokine APRIL (a proliferation-inducing ligand), are recent additions to the tumor necrosis factor (TNF) family (64–69). The discovery of BLyS and the resulting spate of activity aimed at determining its activities have proven to be a critical breakthrough towards understanding the molecular basis for peripheral B-lymphocyte homeostasis, and this work has already been the focus of substantial review and commentary (70–75). Because of its near-simultaneous discovery by several laboratories, BLyS appears in the literature under multiple names: B-cell-activating factor belonging to the TNF family (BAFF) (67), TNF and ApoL-related leukocyte-expressed ligand-1 (TALL-1) (65), TNF homolog-activating apoptosis nuclear factor-κB c-Jun NH2-terminal kinase (THANK) (76), z-TNF-4, and TNF ligand superfamily member 13B (TNFSF13B) (*Table* 4). Herein, the terms BLyS and APRIL are applied for the ligands, and B-cell maturation protein (BCMA) (77, 78), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) (66, 79), and BLyS receptor 3 (BR3) (60, 63), are used for the three receptors, although BR3 is also referred to as BAFF receptor (BAFF-R) (80).

APRIL potentiates B-cell proliferation *in vitro* and B-cell numbers *in vivo* (81). Furthermore, it may play a role in isotype switching during T-independent responses (82), and it appears to act as a costimulatory factor for some T lymphocytes (83). However, because endogenous APRIL fails to rescue B-cell defects in either BLyS- or BR3-deficient mice, a major role in B-cell development and homeostasis seems unlikely. In contrast, compelling evidence indicates that BLyS plays a dominant, lineage-specific role in the selection and homeostatic control of the peripheral B-cell pool. BLyS is generated by monocytes, macrophages, neutrophils, and dendritic cells; it can be induced by a variety of inflammatory cytokines, particularly interferon-γ. Soluble BLyS, which is presently thought to be its only active form, is generated by cleavage and is induced by a growing list of inflammatory substances, including bacterial LPS and some inflammatory cytokines.

Three receptors, all of which are either exclusively or preferentially expressed by B cells, have been described for BLyS and/or APRIL: BCMA (78), TACI (79), and BR3, the product of the previously defined Bcmd locus (30, 63). BLyS interacts with all three of these receptors, whereas APRIL interacts with only TACI and BCMA. The favored interaction of BR3 with BLyS, coupled with recent studies in BR3-signaling mutants, indicate that it is the dominant mediator of BLyS action in peripheral homeostasis and selection (63, 80, 84).

BLyS acts on transitional and mature B lymphocytes

The activities of BLyS and its receptors amongst transitional and mature B cells are summarized in *Fig. 1*. It is unlikely that BLyS plays a role in early B-cell genesis, because neither BLyS binding nor receptor message is found amongst marrow B-cell progenitors prior to the immature B-cell stage (Fr E) (85). Furthermore, neither the size nor dynamics of these early

***Table* 4. BLYS/BLyS receptor family member nomenclature**

Name	Alternative names	Acronym	Reference
APRIL	None	A proliferation-inducing ligand	(69)
BLyS	BLyS	B-lymphocyte stimulator	(64)
	BAFF	B-cell-activating factor of the TNF Family	(67)
	TALL-1	TNF- and ApoL-related leukocyte-expressed ligand-1	(65)
	THANK	TNF homolog that activates apoptosis, NF-kB, and JNK	(76)
	z-TNF-4	z-tumor necrosis factor-4	(66)
	TNFSF20 (later named TNFSF13B)	TNF super family member 20	(123)
BCMA	None	B-cell maturation antigen	(66, 124)
TACI	None	Transmembrane activator and 'calcium-modulating cyclophilin ligand (CAML)' interactor	(66, 125, 126)
BR3	Bcmd	B-cell maturation defect	(30)
	BR3	BLyS receptor 3	(63)
	BAFF-R	BAFF receptor	(80)

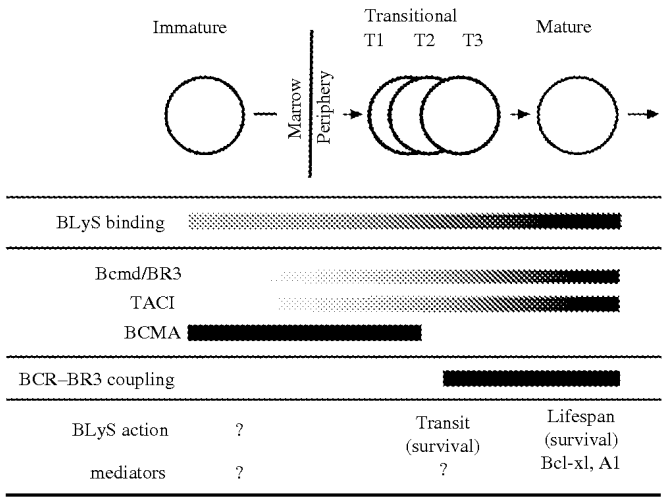

Fig. 1. BLyS responsive stages of B-cell differentiation. Stages of B-cell differentiation that bind BLyS and exhibit BLyS dependence are shown in the top panel. The horizontal bars in the middle three panels depict BlyS-binding capacity, relative expression of each known BLyS receptor, and onset of BCR-coupled BR3 expression. The bottom panel indicates the biological consequences and currently established downstream mediators induced by BLyS.

differentiative stages are altered in BLyS transgenics, BLyS knockouts, BLyS receptor knockouts, or BLyS receptor mutants (30, 62, 86, 87).

In contrast, virtually all B-cell subsets including and subsequent to the immature marrow stage bind BLyS and respond either to BLyS *per se* or to signaling via BLyS receptors. Accordingly, both forced expression and exogenous administration of BLyS yield rapid increases in all but the B1 subset (64). This increase is accompanied by anomalous splenic architecture and elevated levels of serum IgM and IgA. Congruent with these observations, animals treated with the soluble BLyS receptor, TACI-Ig, display a rapid reduction of all mature peripheral pools except the B1 subset (88). Similarly, all mature pools except for the B1 subsets are strikingly reduced in BLyS knockout mice. Finally, as suggested above, Bcmd mutations also yield profound reductions in all peripheral B cells, except the early T1 and B1 subsets (30, 58, 59, 61).

In addition to disproportionately high peripheral B-cell numbers, BLyS transgenic mice display humoral autoimmune symptoms, including serum rheumatoid factors, kidney Ig complex deposition, and a variety of autoantibodies (86, 87). These observations *in toto* suggest that BLyS plays a key role in selective events that normally forestall pathogenic autoreactivity.

BLyS governs successful transitional B-cell differentiation by enhancing survival

The transitional B-cell pools are where specificity-based selection can first be imposed (16, 35, 43). Thus, the finding that excess BLyS not only increases B-cell numbers but also fosters autoantibody formation strongly implied that BLyS influences transitional B cells. In accord with this idea, subsequent findings have clearly established the transitional pools as key targets of BLyS-mediated activity (85, 89, 90). Several lines of evidence show that the receptor primarily responsible for BLyS-mediated effects within transitional cell pools is BR3. First, the *Bcmd* locus, which encodes a mutated form of this receptor, was originally discovered in the A/WySnJ mouse and was named 'B-cell maturation defect' for a phenotype of severely impeded transitional differentiation and inordinately short mature B-cell lifespan (30, 58, 59). Conversely, neither the TACI nor BCMA knockouts exhibit phenotypes with substantially altered transitional B-cell differentiation (91, 92).

Recent studies have probed the effect of BLyS on transitional populations both *in vitro* and *in vivo*. Rolink *et al.* (89) have reported that adding BLyS to cultures of transitional B cells yields extended survival and enables the appearance of mature B cells. These findings led to the suggestion that BLyS provides an inductive signal for maturation (89, 93). However, *in vivo* kinetic analyses revealed that transit rates across peripheral developmental subsets were identical in BLyS-treated and control mice (85), suggesting that prolonged survival, rather than induction of more rapid differentiation, was responsible for enhanced transitional cell differentiation, presumably by altering the proportion of transitional cells surviving to complete maturation in the periphery. This notion has been further explored through analyses of B-cell populations in animals where Bcl-xl expression has been enforced through either transgenic or retroviral transfer approaches (94). In these studies, both the numbers of cells within peripheral subsets as well as the rates at which they are generated are rescued through the enforced expression of Bcl-xl. These findings are most consistent with the idea that survival, rather than an instructive event that engenders differentiation *per se*, underlies the effects of BR3 signaling amongst transitional cells.

The expression of BLyS receptors also differs substantially between each of the transitional subsets and mature B-cell populations (*Fig. 1*) (85). Cells within the T1 compartment predominantly express BCMA. In marked contrast, the T2, T3, and mature follicular populations show the opposite pattern of expression, primarily expressing TACI and BR3 with essentially undetectable levels of BCMA. The basis and significance of this observation remain unclear, although they are consistent with a developmental shift in BLyS responsiveness associated with cells undergoing recruitment into the mature peripheral pool.

Competition for BLyS mediates longevity of mature B cells

The earliest observation suggesting a role for BLyS in maintaining viability amongst mature B cells was the finding that treatment with soluble BLyS receptors resulted in the rapid loss of most mature peripheral B cells (88). Because the lifespan of cells in this pool is approximately 80–120 days (15, 95, 96), the two-week time frame of these reductions was too short to be explained by senescent attrition of mature B cells. Instead, these results were most consistent with the idea that BLyS is required for continued mature B-cell survival.

Studies in the *Bcmd*-mutant mouse strain, A/WySnJ, confirmed and extended this idea in two ways (84). First, *in vivo* BrdU-labeling studies demonstrated that mature B-cell lifespan was severely compromised in the absence of BR3 signaling (30, 61). Second, kinetic studies in (A/WySnJ × BALB/c) F_1 mice showed that B cells heterozygous for the Bcmd have an intermediate but uniform lifespan. These findings are most easily explained by positing that follicular B-cell viability requires continuous signaling via this pathway. In accord with this suggestion, mature A/WySnJ-derived B cells failed to compete effectively for survival in mixed marrow chimeras, because their proportional representation within the mature pool decayed continuously following reconstitution (60, 84). Together, these findings not only established BLyS signaling via the BR3 pathway as the dominant mediator of quiescent peripheral B-cell survival, but also indicated that competition for available BLyS dictates the lifespan of resting mature B cells, thereby mediating homeostatic control of the peripheral B-cell pool.

BLyS promotes viability through the regulation of Bcl-2 family members

In vitro studies from Chen-Kiang and colleagues (97, 98) suggested that a primary activity of BLyS is the attenuation of apoptosis, because BLyS *per se* did not engender mitogenesis but increased the relative representation of several anti-apoptotic Bcl-2 family members (97). The idea that BLyS acts primarily to enhance survival amongst primary B cells *in vivo* was definitively approached through simultaneous kinetic and cell-cycle analyses (85). These experiments showed that BLyS affords increased proportional survival in the late transitional developmental subsets, such that the efficiency of transit to the mature B-cell compartment is increased. Importantly, cell-cycle analyses showed that these effects were engendered by BlyS, without inducing proliferation within any of these subsets.

Members of the Bcl-2 family play a central role in determining the survival and lifespan of normal and neoplastic lymphocytes (reviewed in 99–102). For example, the expression of these genes is regulated during lymphocyte development (103–109), and alterations in Bcl-2 family gene expression can profoundly affect B-lymphocyte survival and differentiation (102, 104, 110–113). The possibility that BLyS might influence the viability and lifespan of B cells through the altered expression of Bcl-2 family members was initially proposed by Do *et al.* (97), who found that in conjunction with CD154 (CD40L), BLyS upregulated anti-apoptotic members of the Bcl-2 family amongst splenic B cells, apparently through a nuclear factor-κB (NF-κB)/relB pathway. Moreover, peripheral blood B cells of BLyS transgenic mice have increased expression of anti-apoptotic Bcl-2 family members. Conversely, an increase in pro-apoptotic Bcl-2 family members has been reported in BLyS receptor mutants (114), further suggesting BLyS-mediated survival might involve the expression of Bcl-2 family genes.

More recently, the influence of BLyS on several Bcl-2 family members was assessed amongst transitional and mature cells following short-term culture with rBLyS (85). These studies showed that mature B cells strongly upregulated both Bcl-xl and A1 in response to BLyS, whereas the upregulation of these genes in bulk transitional populations was low (Fig. 1).

BLyS influences aspects of mature B-cell activation

A role for BLyS in the ligand-driven activation of mature B cells has been posited since early observations that BLyS, in conjunction with mitogenic stimuli, yields enhanced thymidine incorporation. This finding led to an initial interpretation that BLyS serves as a costimulatory factor for B cells, perhaps acting in a fashion similar to CD154 (CD40L), and prompted the suggestion that BLyS might induce proliferation amongst B lymphocytes, leading to the increases in B-cell numbers and Ig levels associated with *in vivo* BLyS treatment. Again, the BR3 receptor appears to be the dominant receptor for this activity, inasmuch as the BR3-mutant A/WySnJ mice do not exhibit enlarged B-cell compartments upon exogenous BLyS administration, and B-cell proliferation assays on $CD23^+$ B cells from these mice fail to display increased thymidine uptake when supplemented with recombinant BLyS (84). Nevertheless, subsequent studies have favored a dominant role for BLyS in mediating survival, rather than any comitogenic activity *per se*, under such stimulatory conditions. Following BCR ligation, there is increased proportional survival within initial daughter cohorts of activated mature B cells, resulting in increased ^{3}H-TdR uptake, without altering division number on a per cell basis (115). If a similar activity exists *in vivo*, it

would suggest that BLyS may determine the proliferative burst size reached by responding primary B-cell clones, which would likely influence the magnitude of primary humoral responses as well as the cells available to initiate germinal centers as memory progenitors. The generally elevated IgM levels in BLyS-treated mice (64) or in BLyS transgenics (86, 87) as well as impaired memory responses in BR3 mutants (59) are consistent with these ideas.

Other aspects of primary follicular B-cell activation may be facilitated or even induced by BLyS signaling as well. In a recent study, Litinski *et al.* (82) demonstrated that dendritic cell-derived BLyS or APRIL could facilitate isotype switching in a CD40-independent manner, if supplemented with appropriate interleukins.

BLyS may influence the formation or maintenance of memory B cells

Although the effects of BLyS on naïve B-cell populations are well documented and accepted, whether BLyS or its receptors play a role in the generation, selection, or maintenance of memory B-cell populations has not yet been addressed in detail. The nature of the A/WySnJ defect at Bcmd has afforded initial interrogation of this possibility, because some mature B cells are indeed formed in these mice and, thus, can be stimulated to participate in immune responses. Ongoing studies in this model indicate that although the BLyS receptor-mutant strain can initiate similar numbers of germinal centers, the characteristic germinal center (GC) enlargement between days 6 and 12 post-immunization fails to occur (60). These results, coupled with earlier studies that showed a relatively normal primary IgM response but unusually low secondary serum responses and diminished IgG titers (59), strongly suggest that BLyS signaling via the BR3 receptor is instrumental in establishing or maintaining humoral memory. Results consistent with the notion that GC initiation is independent of BLyS but that GC persistence is BLyS dependent have recently been reported from the Kalled laboratory (116), in which BlyS-blocking reagents were employed *in vivo*. Similarly, suggesting a role for BLyS in B-cell memory formation or maintenance are observations that BLyS receptor expression shifts amongst different human germinal center subpopulations (117).

BCR signals regulate BR3 expression

It is clear that BLyS plays a pivotal role in the selection, homeostasis, and activation of peripheral B cells. In aggregate, the evidence to date suggests that BLyS controls peripheral B-cell numbers, both by varying the proportion of cells that successfully complete late transitional B-cell development and by serving as the primary determinant of longevity amongst mature follicular B cells. As indicated above, the BCR plays a similar role in B-cell differentiation and survival: immature B cells are targets of BCR-mediated selection, as evidenced by the significant cell losses and repertoire shifts associated with late B-cell maturation; continued BCR expression is requisite for mature B-cell survival; and both BCR specificity as well as altered BCR signaling can dictate relative survival advantage in the periphery.

These conspicuous parallels suggested a potential relationship between BCR signaling and peripheral survival via the BlyS–BR3 pathway, and this possibility has been probed. Recent studies have revealed that BCR ligation upregulates expression of the BLyS receptor BR3 but not other known BLyS receptors. Furthermore, this coupling of BCR signaling with BR3 expression is limited to late transitional and mature B cells.

Are transitional selection and mature B-cell survival reflections of the same process?

It is not yet clear whether the expression of BR3 has an obligate dependence on BCR signaling for expression, or whether a basal level of BR3 signaling is 'switched on' during maturation and subsequently modulated by BCR signaling. Nonetheless, the coupling of BCR signaling with BR3 expression amongst transitional and mature follicular B cells leads to a general hypothesis, whereby specificity-based selection reflects the use of BCR-driven BR3 expression as a determinant of clonal fitness. This model proposes a mechanism, outlined in Fig. 2, whereby the basal BCR-signaling requisite amongst mature B cells affords a necessary minimum level of BR3 expression and that a further survival advantage is afforded clones whose BCRs yield the highest relative levels of BR3. Accordingly, the relative success of transitional differentiation and the lifespan of mature B cells will be dictated by two parameters: available BLyS and relative competitive advantage afforded by BCR-driven BR3 levels. Thus, perturbations that modify either available BLyS levels or the spectrum of competing clonotypes will influence peripheral pool size and composition. It remains possible that both BCR and BR3 signals are simultaneously required for survival, and that BCR driven BR3 levels modulate longevity of clones that minimally meet this dual criterion. This possibility might be tested through enforced BR3 expression in models where BCR

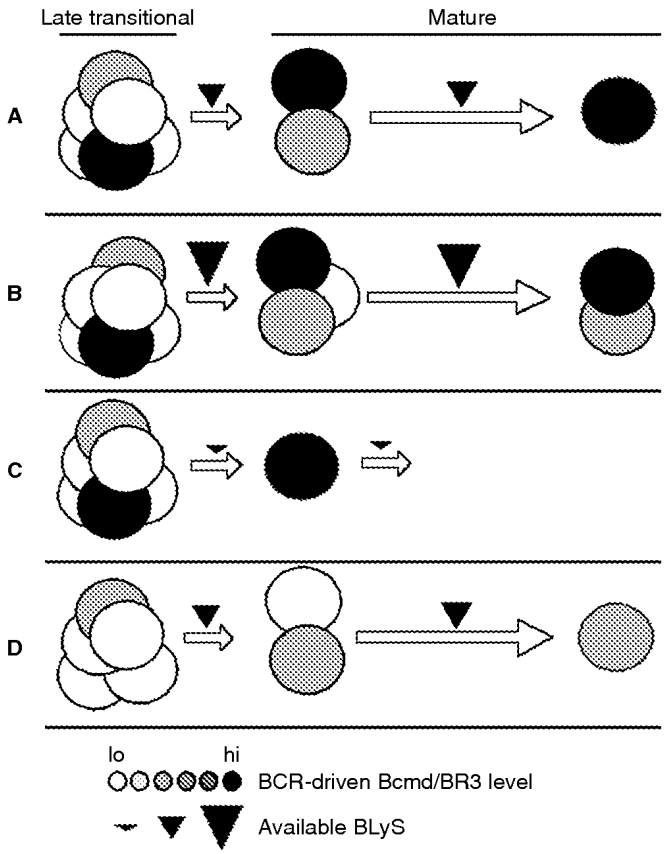

Fig. 2. A unifying model for transitional selection and homeostatic survival. A model whereby transitional selection and homeostatic survival are unified under a single mechanism is depicted, whereby two parameters, available BLyS and BCR-driven BR3 levels, are the primary determinants of transitional success and mature B-cell lifespan. Panel A: Transitional selection and mature lifespan under normal BLyS levels and BCR diversity yield losses of poorly fit clonotypes during transitional differentiation, followed by the protracted competition amongst similarly fit surviving clonotypes. Panel B: Increased BLyS levels afford enhanced transitional differentiation and mature B-cell lifespan, enlarging peripheral pool size. Panel C: Reduced or negligible BLyS levels curtail transitional differentiation and peripheral lifespan, reducing or eliminating peripheral B cells. Panel D: Lack of stringent competition affords maturation and longevity of clonotypes that would otherwise fail or have a short lifespan.

signaling is required for maturation or where the BCR is conditionally ablated after maturation.

This model can be extended to merge specificity-based transitional selection with continuous, specificity-based interclonal competition in the mature repertoire (52, 118). In this scenario, the loss of transitional cells during maturation is mechanistically identical to that underlying interclonal competition amongst mature naïve cells, with the seemingly precipitous losses of transitional cells simply reflecting initial imposition of competitive survival requisites on a previously untested population. Furthermore, it predicts a gradually evolving landscape of specificities, whose intrinsic survival advantage is continuously scrutinized and re-evaluated relative to extant competitor clones. According to this model, continuous selection for clonotypes most fit to capture these signals should lead to a mature primary pool that is less diverse and longer lived, both of which have been reported in aged mice (119–121).

Antigen receptor-coupled lineage-specific cytokine receptors: a paradigm for integrating selection and homeostasis?

A remaining question is whether coupling the expression of lineage-specific cytokine receptors to the antigen and/or pattern recognition receptors characteristic of a functional subset is a general feature of lymphocyte homeostasis. Such a system would afford the homeostatic integration of multiple antigen or pattern recognition receptor signals under the umbrella of a single cytokine, through complimentary or interdigitating cytokine/cytokine receptors sets. Further analyses of differential BLyS receptor expression amongst marginal zone, B1, and memory subsets may yield evidence for such a network.

References

1. Osmond DG. Population dynamics of bone marrow B lymphocytes. Immunol Rev 1986;**93**:103–124.
2. Osmond DG. B cell development in the bone marrow. Semin Immunol 1990;**2**:173–180.
3. Osmond DG, Rolink A, Melchers F. Murine B lymphopoiesis: towards a unified model. Immunol Today 1998;**19**:65–68.
4. Hardy RR, Hayakawa K. B-lineage differentiation stages resolved by multiparameter flow cytometry. Ann N Y Acad Sci 1995;**764**:19–24.
5. Allman D, Li J, Hardy RR. Commitment to the B lymphoid lineage occurs before DH–JH recombination. J Exp Med 1999;**189**: 735–740.
6. Hardy RR, Carmack CE, Shinton SA, Kemp JD, Hayakawa K. Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. J Exp Med 1991;**173**:1213–1225.
7. Hardy RR. B cell ontogeny and B cell subsets. Curr Opin Immunol 1989;**2**:189–198.
8. Li Y-S, Wasserman R, Hayakawa K, Hardy RR. Identification of the earliest B lineage stage in mouse bone marrow. Immunity 1996;**5**:527–535.
9. Hardy RR, Hayakawa K. B cell development pathways. Annu Rev Immunol 2001;**19**: 595–621.
10. Rolink A, Melchers F. B-cell development in the mouse. Immunol Lett 1996;**54**:157–161.
11. Cooper MD. Exploring lymphocyte differentiation pathways. Immunol Rev 2002;**185**:175–185.
12. Hardy RR, Hayakawa K. A developmental switch in B lymphopiesis. Proc Natl Acad Sci USA 1991;**88**:11550–11554.

13. Hardy RR, Carmack CE, Shinton SE, Kemp JD, Hayakawa K. Resolution and characterization of Pro-B and Pre-Pro-B cell stages in normal mouse bone marrow. J Exp Med 1991;**173**:1213–1225.
14. Allman DM, Ferguson SE, Cancro MP. Peripheral B cell maturation I. Immature peripheral B cells in adults are heat-stable antigen^{hi} and exhibit unique signaling characteristics. J Immunol 1992;**149**: 2533–2540.
15. Allman DM, Ferguson SE, Lentz VM, Cancro MP. Peripheral B cell maturation. II. Heat-stable antigen(hi) splenic B cells are an immature developmental intermediate in the production of long-lived marrow-derived B cells. J Immunol 1993;**151**:4431–4444.
16. Carsetti R, Kohler G, Lamers MC. Transitional B cells are the target of negative selection in the B cell compartment. J Exp Med 1995;**181**:2129–2140.
17. Loder F, et al. B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals. J Exp Med 1999;**190**:75–89.
18. Allman D, Lindsley RC, DeMuth W, Rudd K, Shinton SA, Hardy RR. Resolution of three nonproliferative immature splenic B cell subsets reveals multiple selection points during peripheral B cell maturation. J Immunol 2001;**167**:6834–6840.
19. Tyler RW, Everett NB. Radioautographic study of cellular migration using parabiotic rats. Blood 1972;**39**:249–266.
20. Yoshida Y, Osmond DG. Identity and proliferation of small lymphocyte precursors in cultures of lymphocyte-rich fractions of guinea pig bone marrow. Blood 1971;**37**:73–86.
21. Osmond DG, Park YH. B lymphocyte progenitors in mouse bone marrow. Int Rev Immunol 1987;**2**:241–260.
22. Sprent J, Basten A. Circulating T and B lymphocytes of the mouse. II. Lifespan. Cell Immunol 1973;**7**:40.
23. Tough DF, Sprent J. Lifespan of lymphocytes. Immunol Res 1995;**14**:1–12.
24. Tough DF & Sprent J. Turnover of naive- and memory-phenotype T cells. J Exp Med 1994;**179**:1127–1135.
25. Rolink AG, Andersson J, Melchers F. Characterization of immature B cells by a novel monoclonal antibody, by turnover and by mitogen reactivity. Eur J Immunol 1998;**28**:3738–3748.
26. Osmond DG, Wherry PE, Daeron M, Gordon J. Immunoglobulin-bearing cells in bone marrow of mice after prolonged treatment with anti-IgM antibodies. Nature 1976;**260**:328–329.
27. Osmond DG, Gordon J. Characterization of small lymphocytes in the bone marrow of mice after prolonged treatment with anti-IgM antibodies. Cell Immunol 1979;**42**:188–193.
28. Fulop G, Gordon J, Osmond DG. Regulation of lymphocyte production in the bone marrow. I. Turnover of small lymphocytes in mice depleted of B lymphocytes by treatment with anti-IgM antibodies. J Immunol 1983;**130**:644–648.
29. Reid GK, Osmond DG. B lymphocyte production in the bone marrow of mice with X-linked immunodeficiency (xid). J Immunol 1985;**135**:2299–2302.
30. Lentz VM, Cancro MP, Nashold FE, Hayes CE. Bcmd governs recruitment of new B cells into the stable peripheral B cell pool in the A/WySnJ mouse. J Immunol 1996;**157**: 598–606.
31. Norvell A, Monroe JG. Acquisition of surface IgD fails to protect from tolerance-induction. Both surface IgM- and surface IgD-mediated signals induce apoptosis of immature murine B lymphocytes. J Immunol 1996;**156**:1328–1332.
32. Chung JB, Sater RA, Fields ML, Erikson J, Monroe JG. CD23 defines two distinct subsets of immature B cells which differ in their responses to T cell help signals. Int Immunol 2002;**14**:157–166.
33. Su TT, Rawlings DJ. Transitional B lymphocyte subsets operate as distinct checkpoints in murine splenic B cell development. J Immunol 2002;**168**: 2101–2110.
34. Chung JB, Wells AD, Adler S, Jacob A, Turka LA, Monroe JG. Incomplete activation of CD4 T cells by antigen-presenting transitional immature B cells: implications for peripheral B and T cell responsiveness. J Immunol 2003;**171**:1758–1767.
35. Fulcher DA, Basten A. Reduced life span of anergic self-reactive B cells in a double-transgenic model. J Exp Med 1994;**179**: 125–134.
36. Goodnow CC. Transgenic mice and analysis of B-cell tolerance. Annu Rev Immunol 1992;**10**:489–518.
37. Gu H, Tarlinton D, Muller W, Rajewsky K, Forster I. Most peripheral B cells in mice are ligand selected. J Exp Med 1991;**173**: 1357–1371.
38. Levine MH, et al. A B-cell receptor-specific selection step governs immature to mature B cell differentiation. Proc Natl Acad Sci USA 2000;**97**:2743–2748.
39. Turner M, Gulbranson-Judge A, Quinn ME, Walters AE, MacLennan IC, Tybulewicz VL. Syk tyrosine kinase is required for the positive selection of immature B cells into the recirculating B cell pool. J Exp Med 1997;**186**:2013–2021.
40. Cyster JG, Healy JI, Kishihara K, Mak TW, Thomas ML, Goodnow CC. Regulation of B-lymphocyte negative and positive selection by tyrosine phosphatase CD45. Nature 1996;**381**:325–328.
41. Cyster JG. Signaling thresholds and interclonal competition in preimmune B-cell selection. Immunol Rev 1997;**156**:87–101.
42. Sprent J, Bruce J. Physiology of B cells in mice with X-linked immunodeficiency (xid). III. Disappearance of xid B cells in double bone marrow chimeras. J Exp Med 1984;**160**:711–723.
43. Cyster JG, Hartley SB, Goodnow CC. Competition for follicular niches excludes self-reactive cells from the recirculating B-cell repertoire. Nature 1994;**371**:389–395.
44. Cyster JG, Goodnow CC. Antigen-induced exclusion from follicles and anergy are separate and complementary processes that influence peripheral B cell fate. Immunity 1995;**3**:691–701.
45. Cariappa A, Pillai S. Antigen-dependent B-cell development. Curr Opin Immunol 2002;**14**:241–249.
46. Cariappa A, et al. The follicular versus marginal zone B lymphocyte cell fate decision is regulated by Aiolos, Btk, and CD21. Immunity 2001;**14**:603–615.
47. Martin F, Kearney JF. Selection in the mature B cell repertoire. Curr Top Microbiol Immunol 2000;**252**:97–105.
48. Martin F, Kearney JF. Marginal-zone B cells. Nat Rev Immunol 2002;**2**:323–335.
49. Hayakawa K, et al. Positive selection of anti-thy-1 autoreactive B1 cells and natural serum autoantibody production independent from bone marrow B cell development. J Exp Med 2003;**197**:87–99.
50. Hardy RR. Development of VH11^{+} B cells: a model for selection of B cells producing natural autoantibodies. Curr Dir Autoimmun 2003;**6**:196–211.
51. Torres RM, Flaswinkel H, Reth M, Rajewsky K. Aberrant B cell development and immune response in mice with a compromised BCR complex. Science 1996;**272**:1804–1808.
52. Lam KP, Kuhn R, Rajewsky K. In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. Cell 1997;**90**:1073–1083.
53. Bannish G, Fuentes-Panana EM, Cambier JC, Pear WS, Monroe JG. Ligand-independent signaling functions for the B lymphocyte antigen receptor and their role in positive selection during B lymphopoiesis. J Exp Med 2001;**194**:1583–1596.
54. Freitas AA, Rosado MM, Viale AC, Grandien A. The role of cellular competition in B cell survival and selection of B cell repertoires. Eur J Immunol 1995;**25**:1729–1738.
55. McLean AR, Rosado MM, Agenes F, Vasconcellos R, Freitas AA. Resource competition as a mechanism for B cell homeostasis. Proc Natl Acad Sci USA 1997;**94**:5792–5797.

56. De Boer RJ, Freitas AA, Perelson AS. Resource competition determines selection of B cell repertoires. J Theor Biol 2001;**212**:333–343.
57. Agenes F, Rosado MM, Freitas AA. Peripheral B cell survival. Cell Mol Life Sci 2000;**57**:1220–1228.
58. Miller DJ, Hayes CE. Phenotypic and genetic characterization of a unique B lymphocyte deficiency in strain A/WySnJ mice. Eur J Immunol 1991;**21**:1123.
59. Miller DJ, Hanson KD, Carman JA, Hayes CE. A single autosomal gene defect severely limits IgG but not IgM responses in B lymphocyte-deficient A/WySnJ mice. Eur J Immunol 1992;**22**:373.
60. Lentz VM. Bcmd governs recruitment of newly formed B2 cells into the stable peripheral B cell pool. Doctoral Thesis. University of Pennsylvania 1997.
61. Lentz VM, Hayes CE, Cancro MP. Bcmd decreases the life span of B2 but not B1 cells in A/WySnJ mice. J Immunol 1998;**160**:3743–3747.
62. Schiemann B, et al. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. Science 2001;**293**:2111–2114.
63. Yan M, et al. Identification of a novel receptor for B lymphocyte stimulator (BLyS) that is mutated in a mouse strain with severe B cell deficiency. Curr Biol 2001;**11**: 1547–1552.
64. Moore PA, et al. BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. Science 1999;**285**:260–263.
65. Shu HB, Hu WH, Johnson H. TALL-1 is a novel member of the TNF family that is down-regulated by mitogens. J Leukoc Biol 1999;**65**:680–683.
66. Gross JA, et al. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. Nature 2000;**404**:995–999.
67. Schneider P, et al. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. J Exp Med 1999;**189**: 1747–1756.
68. Yu G, et al. APRIL and TALL-I receptors BCMA and TACI: system for regulating humoral immunity. Nat Immunol 2000;**1**:252–256.
69. Hahne M, et al. APRIL, a new ligand of the tumor necrosis factor family, stimulates tumor cell growth. J Exp Med 1998;**188**:1185–1190.
70. Mackay F, Kalled SL. TNF ligands and receptors in autoimmunity: an update. Curr Opin Immunol 2002;**14**:783–790.
71. Mackay F, Browning JL. BAFF: a fundamental survival factor for B cells. Nat Rev Immunol 2002;**2**:465–475.
72. Defrance T, Casamayor-Palleja M, Krammer PH. The life and death of a B cell. Adv Cancer Res 2002;**86**:195–225.
73. Waldschmidt TJ, Noelle RJ. Immunology. Long live the mature B cell – a baffling mystery resolved. Science 2001;**293**: 2012–2013.
74. Laabi Y, Egle A, Strasser A. TNF cytokine family: more BAFF-ling complexities. Curr Biol 2001;**11**:R1013–R1016.
75. Laabi Y, Strasser A. Immunology. Lymphocyte survival – ignorance is BLyS. Science 2000;**289**:883–884.
76. Mukhopadhyay A, Ni J, Zhai Y, Yu GL, Aggarwal BB. Identification and characterization of a novel cytokine, THANK, a TNF homologue that activates apoptosis, nuclear factor-κB, and c-Jun NH2-terminal kinase. J Biol Chem 1999;**274**:15978–15981.
77. Laabi Y, Gras MP, Brouet JC, Berger R, Larsen CJ, Tsapis A. The BCMA gene, preferentially expressed during B lymphoid maturation, is bidirectionally transcribed. Nucleic Acids Res 1994;**22**:1147–1154.
78. Madry C, et al. The characterization of murine BCMA gene defines it as a new member of the tumor necrosis factor receptor superfamily. Int Immunol 1998;**10**:1693–1702.
79. von Bulow GU, Bram RJ. NF-AT activation induced by a CAML-interacting member of the tumor necrosis factor receptor superfamily. Science 1997;**278**:138–141.
80. Thompson JS, Browning S, Ambrose C. BAFF-R, a novel TNF receptor that specifically interacts with BAFF. Science 2001;**293**:2108–2111.
81. Rennert P, et al. A soluble form of B cell maturation antigen, a receptor for the tumor necrosis factor family member APRIL, inhibits tumor cell growth. J Exp Med 2000;**192**:1677–1684.
82. Litinskiy MB, et al. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat Immunol 2002;**3**:822–829.
83. Stein JV, et al. APRIL modulates B and T cell immunity. J Clin Invest 2002;**109**: 1587–1598.
84. Harless SM, et al. Competition for BLyS-mediated signaling through Bcmd/ BR3 regulates peripheral B lymphocyte numbers. Curr Biol 2001;**11**:1986–1989.
85. Hsu BL, Harless SM, Lindsley RC, Hilbert DM, Cancro MP. Cutting edge: BLyS enables survival of transitional and mature B cells through distinct mediators. J Immunol 2002;**168**:5993–5996.
86. Khare SD, et al. Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. Proc Natl Acad Sci USA 2000;**97**:3370–3375.
87. Mackay F, et al. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. J Exp Med 1999;**190**:1697–1710.
88. Gross JA, et al. Taci-Ig neutralizes molecules critical for B cell development and autoimmune disease: impaired B cell maturation in mice lacking BLyS. Immunity 2001;**15**:289–302.
89. Rolink AG, Tschopp J, Schneider P, Melchers F. BAFF is a survival and maturation factor for mouse B cells. Eur J Immunol 2002;**32**: 2004–2010.
90. Batten M, et al. BAFF mediates survival of peripheral immature B lymphocytes. J Exp Med 2000;**192**:1453–1466.
91. Xu LG, Wu M, Hu J, Zhai Z, Shu HB. Identification of downstream genes up-regulated by the tumor necrosis factor family member TALL-1. J Leukoc Biol 2002;**72**:410–416.
92. Yan M, et al. Activation and accumulation of B cells in TACI-deficient mice. Nat Immunol 2001;**2**:638–643.
93. Rolink AG, Melchers F. BAFFled B cells survive and thrive: roles of BAFF in B-cell development. Curr Opin Immunol 2002;**14**:266–275.
94. Amanna IJ, Dingwall JP, Hayes CE. Enforced bcl-xL gene expression restored splenic B lymphocyte development in BAFF-R mutant mice. J Immunol 2003;**170**: 4593–4600.
95. Osmond DG. The turnover of B cell populations. Immunol Today 1993; **14**:34–37.
96. Sprent J, Tough DF. Lymphocyte life-span and memory. Science 1994;**265**: 1395–1400.
97. Do RK, Hatada E, Lee H, Tourigny MR, Hilbert D, Chen-Kiang S. Attenuation of apoptosis underlies B lymphocyte stimulator enhancement of humoral immune response. J Exp Med 2000;**192**:953–964.
98. Do RK, Chen-Kiang S. Mechanism of BLyS action in B cell immunity. Cytokine Growth Factor Rev 2002;**13**:19–25.
99. Adams JM, et al. Control of apoptosis in hematopoietic cells by the Bcl-2 family of proteins. Cold Spring Harb Symp Quant Biol 1999;**64**:351–358.
100. Cory S. Regulation of lymphocyte survival by the bcl-2 gene family. Annu Rev Immunol 1995;**13**:513–543.
101. Korsmeyer SJ. Regulators of cell death. Trends Genet 1995;**11**:101–105.
102. Strasser A, et al. Lessons from Bcl-2 transgenic mice for immunology, cancer biology and cell death research. Behring Institute Mitteilungen 1996;101–117.
103. Gibson LF, Piktel D, Narayanan R, Nunez G, Landreth KS. Stromal cells regulate bcl-2 and bax expression in pro-B cells. Exp Hematol 1996;**24**:628–637.

104. Grillot DA, et al. Bcl-x exhibits regulated expression during B cell development and activation and modulates lymphocyte survival in transgenic mice. J Exp Med 1996;**183**:381–391.
105. Li YS, Hayakawa K, Hardy RR. The regulated expression of B lineage associated genes during B cell differentiation in bone marrow and fetal liver. J Exp Med 1993;**178**: 951–960.
106. Liu YJ, et al. Germinal center cells express bcl-2 protein after activation by signals which prevent their entry into apoptosis. Eur J Immunol 1991;**21**:1905–1910.
107. Merino R, Ding L, Veis D, Korsmeyer S, Nunez G. Developmental regulation of the Bcl-2 protein and susceptibility to cell death in B lymphocytes. EMBO J 1994;**13**: 683–691.
108. Nunez G, Merino R, Grillot D, Gonzalez-Garcia M. Bcl-2 and Bcl-x: regulatory switches for lymphoid death and survival. Immunol Today 1994;**15**:582–588.
109. Tomayko MM, Cancro MP. Long-lived B cells are distinguished by elevated expression of A1. J Immunol 1998;**160**:107–111.
110. McDonnell TJ, et al. Bcl-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. Cell 1989;**57**:79–88.
111. O'Reilly LA, Harris AW, Tarlinton MD, Corcoran LM, Strasser A. Expression of a bcl-2 transgene reduces proliferation and slows turnover of developing B lymphocytes in vivo. J Immunol 1997;**159**:2301–2311.
112. Strasser A, Harris AW, Corcoran LM, Cory S. Bcl-2 expression promotes B- but not T-lymphoid development in scid mice. Nature 1994;**368**:457–460.
113. Strasser A, et al. Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. Proc Natl Acad Sci USA 1991;**88**: 8661–8665.
114. Clise-Dwyer K, Amanna IJ, Duzeski JL, Nashold FE, Hayes CE. Genetic studies of B-lymphocyte deficiency and mastocytosis in strain A/WySnJ mice. Immunogenetics 2001;**53**:729–735.
115. Smith SH, Cancro MP. Cutting edge: B cell receptor signals regulate BLyS receptor levels in mature B cells and their immediate progenitors. J Immunol 2003;**170**: 5820–5823.
116. Vora KA, et al. Germinal centers formed in the absence of B cell-activating factor belonging to the TNF family exhibit impaired maturation and function. J Immunol 2003;**171**:547–551.
117. Avery DT, et al. BAFF selectively enhances the survival of plasmablasts generated from human memory B cells. J Clin Invest 2003;**112**:286–297.
118. Rosado MM, Freitas AA. The role of the B cell receptor V region in peripheral B cell survival. Eur J Immunol 1998;**28**: 2685–2693.
119. Posnett DN, et al. Oligoclonal expansions of antigen-specific CD8+ T cells in aged mice. Ann N Y Acad Sci 2003;**987**:274–279.
120. Kline GH, Hayden TA, Klinman NR. B cell maintenance in aged mice reflects both increased B cell longevity and decreased B cell generation. J Immunol 1999;**162**:3342–3349.
121. Johnson KM, Owen K, Witte PL. Aging and developmental transitions in the B cell lineage. Int Immunol 2002;**14**:1313–1323.
122. Melchers F, et al. Positive and negative selection events during B lymphopoiesis. Curr Opin Immunol 1995;**7**:214–227.
123. Tribouley C, et al. Characterization of a new member of the TNF family expressed on antigen presenting cells. Biol Chem 1999;**380**:1443–1447.
124. Gras MP, et al. BCMAp: an integral membrane protein in the Golgi apparatus of human mature B lymphocytes. Int Immunol 1995;**7**:1093–1106.
125. von Bulow GU, Russell H, Copeland NG, Gilbert DJ, Jenkins NA, Bram RJ. Molecular cloning and functional characterization of murine transmembrane activator and CAML interactor (TACI) with chromosomal localization in human and mouse. Mamm Genome 2000;**11**:628–632.
126. Xia X, et al. TACI is a TRAF-interacting receptor for TALL-1, a tumor necrosis factor family member involved in B cell regulation. J Exp Med 2000;**192**:137–143.